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TRANSMISSION OF MICROSPORIDIAN PARASITES OF MOSQUITOES.(U)
JAN 81 D W HALL, E I HAZARD N00014-80-C-0172

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Studies were conducted on dimorphic microsporidian parasites of the mosquitoes *Culex peccator*, *Culex pilosus*, *Culiseta inornata*, *Aedes taeniorhynchus*, and *Deinocerites cancer*. The parasites of *C. pilosus* and *D. cancer* are unusual in that two types of spores (presumably haploid and diploid) are found simultaneously in the same tissue. Preliminary evidence suggests that the parasite in *C. pilosus* may be infectious *per os*.

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20. ABSTRACT (continued)

The parasite of *C. inornata* forms haploid spores in the gastric caeca of late instar larvae. An asexual sequence in adult females leads to infection of developing eggs where diploid spores may be observed.

The dimorphic microsporidium which was studied in *Aedes taeniorhynchus* is characterized by a fringed exospore and lack of an apparent pansporoblast membrane. This parasite is vertically transmitted for only a single generation. Therefore all other transmission must be horizontal.

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ANNUAL REPORT NO. 1

Transmission of Microsporidian Parasites of Mosquitoes*

by

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Introduction

Microsporidia of the family Thelohaniidae are common parasites of mosquitoes and certain other invertebrates. They have complex life cycles and exhibit dimorphic development. In the adult female host the parasite forms small numbers of single binucleate spores which serve to infect the developing oocytes resulting in transovarial (vertical) transmission to the progeny of the infected female. Some species of microsporidia are transmitted in this manner for many generations while others are vertically transmitted for only 1 generation and all infected progeny die prior to reaching reproductive age. In both types of parasites a different type of spore is formed in the progeny than that formed in the infected female. These spores are uninucleate and packaged in groups of eight within a membrane. These uninucleate spores do not appear to be infectious when fed directly to mosquitoes.

We have shown that vertical transmission alone is not sufficient for maintenance of at least some of these parasites in nature. However, at the present time, none of these parasites have been successfully transmitted in the laboratory except by vertical transmission.

This contract is concerned with the microsporidian genera *Amblyospora* and *Parathelohania* and certain species of other genera which have dimorphic life cycles and are transovarially transmitted in mosquitoes. The primary objectives of this research are to work out the life cycles of these parasites and to determine the mechanism of horizontal transmission of the parasites from mosquito to mosquito.

Most of the work this year has been devoted to elucidation of life cycles of different species of dimorphic microsporidia. Some interesting variations in life cycles have been discovered, but so far these studies

have not yielded additional clues on the mechanism of horizontal transmission.

Materials and Methods

Experimental Animals. - *Culex peccator* mosquitoes were provided by Dr. Harold C. Chapman, Gulf Coast Mosquito Research Laboratory, Lake Charles, Louisiana. *Culiseta inornata* were field-collected in Alachua County, Florida. *Deinocerites cancer* and *Aedes taeniorhynchus* were field-collected from Dade County, Florida and Collier County, Florida, respectively.

Adult mosquitoes were maintained at approximately 24° C under natural photoperiod and were constantly supplied with a 5% sucrose solution. Females were also offered guinea pigs or chicks (in the case of *Deinocerites*) as a blood source for development of eggs.

Life Cycle Studies. - To determine the developmental sequences and life cycles of the parasites, the mosquito hosts were sequentially sacrificed at different stages of development and examined to determine the developmental stage of the parasite.

General characterization of the microsporidian stages at the light microscope level was made from Giemsa-stained smears of infected host tissues, as described by Hazard and Oldacre (2). Sites of infection within the mosquito host were determined from whole mosquitoes fixed in Carnoy's solution, embedded in paraffin, sectioned at 6 µm, and stained with iron hematoxylin and eosin Y.

For ultrastructural studies, infected specimens were dissected in 2.5% (w/v) gluteraldehyde buffered with 0.1 M sodium cacodylate (pH 7.5) and fixed for 2 h at room temperature, in the dark in 2.5% gluteraldehyde, 0.1% (v/v) peroxide in 0.1 M cacodylate buffer, pH 7.5 (3). After several buffer washes, specimens were postfixed in 1% (w/v) OsO_4, dehydrated in an ethanol series, *en bloc* stained with 0.5% (w/v) uranyl acetate in 70% ethanol and embedded either Spurrs (5), a Spurr-Epon mixture (1), or

Epon-Araldite. Sections were poststained with 5% (w/v) methanolic uranyl acetate, followed by lead citrate (4) and examined in a Hitachi HU-125 E electron microscope at an acceleration voltage of 75 kV.

Results and Discussion

One of the parasites we have studied is a species of *Amblyospora* in the mosquito *Culex peccator*. This parasite is transmitted to all or most of the progeny of an infected female. It is somewhat unique among the Amblyosporidae in that the developmental sequence which leads to the production of haploid spores takes place in perikarya of the neurons of the brain and ganglia of the ventral nerve cord of the mosquito larvae. Spores are formed as early as the second instar and continue to be formed throughout the third and fourth instars. Most larvae with patent infections survive, but a few with extensive infections have severe damage to the nervous system and succumb to the infection. The other developmental sequence which leads to transovarial transmission has not yet been worked out. However, diplokaryotic stages have been observed in Giemsa-stained smears of adult female *C. peccator*.

Culiseta inornata infected with *Pleistophora caecorum* was colonized in order to study the life cycle of the microsporidium in the mosquito host. The microsporidium was found to be a dimorphic form with one sequence of its development forming haploid spores following meiotic divisions in young sporonts. These haploid spores are formed in the gastic caeca of both male and female (3rd and 4th instar) larvae. Another sequence develops asexually in females, invades their ovaries, and forms sporonts, sporoblasts, and finally spores which may be observed in smears of eggs. These spores which are diploid have been seen in the guts of newly hatched

larvae. It is possible that this may be a case of *per os* transmission. If so, it may lend some credence to the possibility of the diploid spores functioning in horizontal transmission.

Another microsporidium which is currently being studied is a rather unusual dimorphic species which parasitizes the black salt-marsh mosquito *Aedes taeniorhynchus*. The parasite is transovarially transmitted for one generation only. Cylindrical spores in adult female mosquitoes pass the infection to males of the next generation. In larvae, meronts with one or two diplokarya give rise to sporonts with two, four, six, or eight nuclei. No pansporoblastic membrane is evident. Spores in larvae, pupae, and adult males are uninucleate, short pyriform, and fringed at the exospore. In most specimens, few spores are formed until the mosquito reaches the adult stage. Development of this parasite is similar to the dimorphic Thelohaniidae except for the shape of the spores in males and the apparent lack of a pansporoblastic membrane. It is believed that the spores found in males are haploid and we are currently examining this parasite for evidence of meiosis. Attempts at transmission utilizing the pyriform spores have met with failure.

We have also recently discovered a new dimorphic microsporidium in the mosquito *Deinocerites cancer*. This species forms two spore types in the same tissue (fat body). We have not yet attempted to transmit this species.

One of us (E.I.H.) has recently made an interesting discovery with *Stempellia lunata* in *Culex pilosus*. This species produces both haploid and diploid spores in larvae, and preliminary experiments suggest that this parasite may be infectious when fed to healthy larvae. More work is needed on this species to confirm these results and to see how this parasite is transmitted transovarially. It is obviously quite different than most other dimorphic microsporidia of mosquitoes, since in most species the

haploid spores are not infective for mosquitoes and the diploid spores are generally considered to function in transovarial transmission only.

Crayfish and freshwater shrimp have been examined as possible candidate alternate hosts. A survey of 6 species show that they also harbor dimorphic microsporidia. Species of *Thelohania* were found in *Cambarellus puer, C. schmitti, Palaemonetes paludosa,* and *Procambarus paeninsulanus.* Dimorphic *"Pleistophora"* were found in *Palaemonetes paludosa, Procambarus fallax,* and *P. lucifugus alachuae.*

We have now also established a large laboratory colony of *Amblyospora*-infected *Culex salinarius.* We are mass-producing and storing the haploid spores of this species. These spores will be used during 1981 to produce highly specific monoclonal antibodies by the hybridoma technique. These antibodies will then be utilized in mass screening for candidate intermediate hosts for *Amblyospora.* The enzyme-linked immunosorbent assay will be used to attempt to detect *Amblyospora* antigens in the candidate intermediate hosts. Any of the species reacting positively with the hybridoma antibodies will then be fed haploid and diploid spores from mosquitoes in an attempt to complete the cycle.

Life cycle studies of the dimorphic microsporidia in mosquitoes are also being continued.

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